THE GLYCEMIC RESPONSE TO AMINO ACID INFUSION IN INFANTS OF DIABETIC MOTHERS

Robert F. Taylor

1976

YALE

MEDICAL LIBRARY

THE GLYCEMIC RESPONSE TO AMINO ACID INFUSION IN INFANTS OF DIABETIC MOTHERS

ROBERT F. TAYLOR
B.S., University of Wisconsin, 1972

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Medicine

YALE UNIVERSITY
March 1, 1976

CONTENTS

To my parents.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the following people for their assistance:

Ms. Barb Smith and the nurses of the Newborn Special Care Unit for putting up with me over the course of the past two years.

Mary Terry for invaluable technical assistance and encouragement for the perfection of the alanine assay.

Eight very special infants without whose cooperation this study would have been impossible.

My sister Kathy for the many hours she spent wading through my scribbled manuscript and converting it into a professional final form.

Dr.Ricardo Uauy for his generous support and assistance in every aspect of this study. His fascile mind, quick wit, and "one-stick" venipunctures made it a real pleasure to work with him. Our loss will be Chile's gain.

Finally, Dr. Joseph B. Warshaw, my advisor, for his steadfast support throughout my years in medical school. His criticisms, encouragement, and a fund of knowledge that continues to amaze me have made this project truly memorable for me. I am honored to have him as a friend and advisor.

"In all abundance there is lack"

- Hippocrates

INTRODUCTION

The infant of the diabetic mother is one of the most intensively studied yet enigmatic subjects in pediatrics. The primary focus of the investigations of these infants has been directed toward their tendency to become hypoglycemic in the immediate post partum period. From the time of Hartmann and Jaudon's article in 1937 (1) through the present, inquiries into the frequency and significance of neonatal hypoglycemia have been abundant. While there have been reports which have denied the predilection for hypoglycemia in the IDM (2), the current view of the situation is that approximately one half of all infants born to diabetic mothers experience a fall in blood glucose levels to below 20 mg/100 ml within the first few hours of life (3,4).

The significance of the fall in glucose has been debated. Several authors have suggested that neonatal hypoglycemia is of no clinical significance (5,6). However, a study by Beard et al (7) has shown that there is, in fact, a significant incidence of brain damage observed in infants who experience neonatal hypoglycemia. Additionally, animal models (8) have demonstrated that anabolic processes seem to be interfered with during periods of hypoglycemia. Studies of the human brain in insulin coma have shown that there is a decreased oxygen uptake during the hypoglycemic period (9). A follow-up

study of 151 children concluded that symptomatic hypoglycemia in newborns was associated with CNS symptoms and a poor prognosis, while asymptomatic hypoglycemia was not associated with significant morbidity later in life (10). This data has led most investigators to believe that it is worthwhile maintaining neonatal normoglycemia or at least to prevent the profound hypoglycemia to which IDM's appear prone.

The cause of the neonatal hypoglycemia is uncertain. For some time it was felt that the relative hypoglycemia during pregnancy induces islet cell hyperplasia in the infant of the diabetic, and that this hyperplasia in turn is responsible for a relative or absolute hyperinsulinism postnatally. The theory, first proposed in the 1920's by Dubreuil and Anderodias, has been supported over the years by observations that infants of diabetic mothers have shown islet cell hyperplasia and hypertrophy (11), increased amounts of body fat (12), higher birth weights (13), a failure to increase their free fatty acids in the neonatal period (14), and finally, neonatal hypoglycemia.

Pederson and others have hypothesized that the hypoglycemia of these infants is, in fact, due to a hyperinsulinism. In support of this theory is evidence which has been accumulated which shows that IDM's demonstrate a more rapid disappearance of endogenous glucose if tested

shortly after birth (15) and that when exogenous glucose is administered to these infants in the form of an intravenous glucose loading test, they also show a more rapid decrease than do normal infants (16).

In opposition to this theory, one must recognize that although insulin levels may be increased in infants of diabetic mothers, blood sugar levels are also increased. The difference between insulin values in infants of diabetics and normals disappeared after the age of 2 hours (17) coincident to the return of IDM blood sugar levels to the range of normals. Furthermore, several workers have shown that insulin release is not increased after slow IV glucose loads in IDM's even though they show the aforementioned rapid glucose disappearance under other circumstances (18).

In the present study, we have attempted to assess the role of the amino acids alinine in the hypoglycemia of neonatal IDM's. Van Slyke and Meyer were the first to notice that in a condition of negative nitrogen balance, the muscles of animals release free amino acids. By their studies in dogs which had been fasted for some time, they concluded that muscle tissue protein is the source of amino acids (19). Since that time, a great deal of work on the mechanisms of alanine release has been done. This work culminated in the work of Felig (20) in which he postulates "the glucose-alanine cycle."

In brief, this theory proposes that alanine acts as the substrate for hepatic gluconeogenesis. Since that study appeared, much work has been done in the elucidation of more precise mechanisms involved in glucostasis. Of particular interest to the present study is the work that has been done with diabetic patients. It has been shown, for example, that the level of alanine is markedly reduced in diabetics. This low level returns towards normal with the administration of insulin (21-23). This and other observations have led to the hypothesis that the hyperglycemia seen in diabetes is a function of increased hepatic extraction rather than increased substrate availability (23). Increases in splaunchnic uptake of alanine have been observed in diabetics, accounting for approximately 50% of the glucose output in these individuals (22). This increased splaunchnic uptake and therefore, hepatic glucose output may be a key factor in hyperglycemia associated with diabetes.

As precarious as our understanding of the homeostatic mechanisms concerning glucose is in the adult, it is still more tenuous in the case of the newborn. As was mentioned previously, the separation of the fetus from the placental source of nutrients results in a cascade of events, primary among them a change in the glucose production-utilization balance (24). Studies by Cahill (25) and others (26) have implicated glucagon as a necessary participant in the activation of glycogenolysis and gluconeogensis in the

newborn. The observation that glucagon and glucose bear an inverse relationship to one another in the newborn period (27) led some of the early studies to suggest that similar relationships as those seen in the adult occur routinely in the newborn period. Sperling et al (28) found that in normal newborns, glucagon secretion can be stimulated by alanine infusion. Other studies showed that infusion of alanine into the pregnant mother in labor produced higher levels of glucagon and glucose in the cord blood (29) when compared to similar results of women given saline infusions.

Administration of alanine intravenously to newborn infants in one study produced a rise in glucose levels, but of small magnitude (30). In another study, results of alanine infusion were compared between groups of newborns and adults. Adults given an alanine infusion showed significant increases in serum alanine levels, but failed to show an increase in glucose. In contrast, marked increases in glucose (36 - 137 mg %) occurred in half of the normal and 1 small for gestational age infant at 6 hours of age. This increase was associated with similar elevations of alanine, lactate, and pyruvate levels. By 36 hours of age, all term newborns showed an increase in glucose of 12% in response to alanine infusion. Pyruvate and lactate levels rose 20-50% following the infusion (31). These results led the investigators to postulate that alanine is used for gluconeogenesis in the newborn, but

that it may be incomplete, with some conversion to other products.

Because of their tendency toward hypoglycemia (31), small for gestational age infants have also been studied with respect to alanine metabolism. Williams et al found that small for gestational age infants had higher basal levels of alanine that control newborns (31) and that oral alanine feeding produced significant rises in glucagon and alanine levels in both groups. However, only the appropriate for gestational age infants showed consistent increases in glucose and insulin in response to alanine. In infants over 24 hours of age, alanine feedings had no effect in either group on levels of glucagon, insulin or glucose. The authors attributed these different responses to decreased glycogen stored and/or inadequate gluconeogenic enzymes in the SGA infants. These studies were subsequently confirmed by another group using intravenous techniques of alanine administration (33). In addition to this work, a study by Haymond and coworkers examined glucose, lactate, amino acids, and several other substances in SGA infants. They found significant inverse correlations between glucose and lactate and glucose and alanine at two hours of age (32). Indeed, all potential gluconeogenic amino acids were found to be significantly elevated in these infants.

To date there has been no reported attempt to ascertain the effect of alanine on infants of diabetic mothers. It is the purpose of the present study to investigate the effects

of an intravenous alanine load on infants of diabetic mothers. It is postulated that part of the mechanism involved in the neonatal hypoglycemia often noticed in these infants may be related to an insensitivity or relative insensitivity to substrate availability. Additionally, we have sought to establish temporal differences in IDM response to an alanine load which would correlate with either development of the neonatal regulatory mechanisms or to the disappearance of maternal environmental antagonists.

MATERIALS AND METHODS

A total of eight infants were included in the study group. All study procedures had received prior approval by our hospital's Committee on Human Investigation. Infants to be included in the study were selected on the basis of evidence of maternal diabetes. A mother was considered for the study if a) she demonstrated an abnormal glucose tolerance test during her pregnancy, or b) she had a documented history of diabetes mellitus, either insulin or diet controlled. These descriptions correspond to White's classifications A and B (34). No infants of Class C-F diabetics were considered. There were no significant differences in birth weights or gestational ages between the two groups. All infants were between 38 and 40 weeks by dates. All infants in the study group were delivered vaginally, and all were in a vertex presentation at the time of birth.

In addition to the screening process above, infants of Class A or gestational diabetic mothers were further evaluated after birth to assess their suitability for inclusion in the study. Only those infants demonstrating characteristics of IDM's were included in the study (35).

Infants accepted for participation in the study were divided into two groups on a random basis, without regard to size, gestational age, or degree of maternal diabetes. The first group was studied at three hours of age. The

second group was studied at 12 hours of age. In the group studied at 12 hours of age, the infants were tested following a four hour fast. The early group was studied prior to the first feeding.

Infants included in the study group were observed closely for signs of hypoglycemia and other neonatal complications. Any infant developing hypoglycemia (Dextrostix less than 45) was fed immediately and removed from the study group. Furthermore, any infant developing signs of neonatal distress (tachypnea, grunting, retractions, jaundice, etc.) was treated for that condition and removed from the study group. Infants to be studied at 12 hours of age were placed on a normal feeding schedule until four hours prior to the time of study. Any infant developing hypoglycemia during the four hour fast was removed from study.

Prior to alanine infusion, blood was withdrawn from a heelstick or peripheral vein for baseline levels of alanine, insulin, glucose, and glucagon. Infants were then given a bolus injection of l-alanine (125 mg/kg) as a 10% solution. The solution was administered over a period of 3 minutes through a peripheral vein. The alanine solution was made up in our hospital pharmacy and underwent sterility and USP pyrogen testing prior to use.

Following injection of l-alanine, blood samples were withdrawn from a peripheral vein or heelstick at 15, 30, and 60 minutes. Blood to be analyzed for glucagon was immediately placed in chilled tubes containing EDTA (10.4 mg)

and Trasylol (1000 kallikrein inhibitor units, FBA Pharmaceuticals, New York). Samples to be analyzed for insulin were placed in chilled heparinized tubes (286 units USP heparin). Both sets of samples were refrigerated and centrifuged, the plasma removed for analysis. Plasma was stored at -50 degrees C until assayed. Blood for glucose was centrifuged immediately and the plasma removed. Blood to be analyzed for alanine was centrifuged, the plasma removed, and frozen at -50 degrees C until assayed. The total amount of blood removed from the infants did not exceed 15 ml over the course of the study.

ASSAYS

Glucagon and Insulin: Glucagon and insulin assays were performed for us by Dr. Philip Felig's laboratory in the section of Endocrinology and Metabolism, using a radioimmunoassay. The samples we submitted were processed along with regular clinical specimens and controlled using normal control procedures. Glucose: Glucose was measured in our hospital's clinical chemistry lab using their standard automated glucose-oxidase system. All samples were run in duplicate.

ALANINE

Alanine was measured in our lab using a microfluorometric technique modified after Lowry's method.

Reaction sequence:

1) alanine + ketoglutarate $\xrightarrow{\text{GPT}}$ pyruvate + glutamate

LDH
2) pyruvate + NADH + H ----- lactate + DPN

Materials: alpha ketoglutarate, alanine (grade III), reduced nicotinamide adenine dinucleotide (NADH), rabbit muscle lactic dehydrogenase (LDH, type III, crystalline in ammonium sulfate suspension), pig heart glutamic-pyruvic transaminase (GPT, suspension in 1.8 M ammonium sulfate solution), and tris (hydroxymethyl) aminomethane were all purchased from the Sigma Chemical Company. Potassium hydroxide and perchloric acid were purchased from the Fisher Scientific Company.

All glassware for this assay was cleaned following Lowry's method, using a solution of concentrated nitric acid followed by rinsing in distilled water (high resistance greater than 1 meg ohm with low fluorescence background). The test tubes used to hold the intermediate solutions were standard 10 x 75 mm glass test tubes. The fluorometric readings were made using quartz cuvettes cleaned in the above manner. Readings were made using a Perkin Elmer Hitachi flucrometer. All distilled water for this assay was purified as above and periodically tested for purity.

Stock solutions of alanine, alpha keto glutarate (1.46 g/ 100 ml distilled water), NADH (7 mg/ 10 ml distilled water), were made fresh immediately prior to the assay. Standard solutions of 3N perchloric acid, 25 mM Tris (pH 8.1), and 3N potassium hydroxide were made up at the beginning of the

study and kept refrigerated throughout the study. Solutions of LDH, and GPT were made up immediately prior to their addition to the reaction mixture. All solutions were pipetted using standard Corning glass pipettes.

Methods: Samples obtained and stored as described previously were removed from the freezer and allowed to stand at room temperature until they had thawed completely. After the samples were mixed thoroughly, 100 microliters of serum was removed from each specimen and placed in a clean 10 x 75 mm test tube. The remainder of the serum was immediately returned to the freezer for later use. To the serum was added 350 microliters of chilled distilled water and 50 microliters of ice cold 3N perchloric acid. The tubes were then mixed gently on a Vortex mixer until thoroughly mixed, placed on ice, and allowed to settle for 30 minutes.

At the end of this time, the specimens were placed in a centrifuge at 0 degrees C and spun at 500 g for an additional 20 minutes. 300 microliters of the supernatant obtained from these samples was removed and placed in labeled 10 x 75 mm test tubes. To this sample was added 330 microliters of chilled distilled water and 29 microliters of ice cold 3N potassium hydroxide. This mixture was again mixed gently on a Vortex mixer and allowed to settle for 30 minutes. At the end of this time, the pH of each tube was checked and adjusted to a final pH of between 6 and 7. This was done by adding small aliquots of the stock KOH

solution to the samples. The samples were then placed in the centrifuge and spun for an additional 20 minutes to settle the precipitate. The resulting supernatant was then removed for analysis.

Clean cuvettes were check again for contaminants, following which, 100 microliters of supernatant was placed in each of the cuvettes. To this quantity was added 2.22 ml of Tris solution, .03 ml NADH, 0.6 ml of alpha keto-glutarate. The cuvettes were then placed in the fluoro-meter and an initial reading was made. In those cuvettes containing alanine standards, the supernatant was replaced by an equal amount of alanine of known concentration. Each run also contained a blank. After the initial reading had been made, 0.7 units of lactic dehydrogenase was added to the cuvettes at 5 second intervals. These were, in turn, mixed thoroughly and allowed to stand for 20 minutes at room temperature. The cuvettes were then placed in the fluoro-meter and readings were made in the same order as above, with 5 second intervals in between readings.

After the readings had been recorded, .075 ml of GPT was added, in order, to each of the cuvettes, which were mixed thereafter. The samples were then placed in a water bath at 37 degrees C for 45 minutes. At the end of this period, the cuvettes were removed from the water and rinsed in distilled water several times and dried completely. They

were allowed to stand at room temperature for an additional 25 minutes, at the end of which time, a final reading was made of each cuvette in the same order.

Fluorometric readings were translated from a standard curve constructed earlier and standard concentrations of alanine were compared for accuracy with each run. Samples were run in batches and in duplicate. Test results from this method were checked prior to the assay of actual samples with the results obtained using an amino acid analyzer in Dr. P. Felig's laboratory. A good correlation was obtained between these data, Dr. Felig's results, and the known concentrations of the test samples.

STATISTICS

All statistics were analyzed for paired, grouped, and student analysis where appropriate. All data was compiled and analyzed on a Hewlett-Packard model 9830 A computer programmed for such calculations.

RESULTS

The study group was divided into early (3 hours) and late (12 hours) subgroups. All infants studied were either offspring of Class A or Class B diabetic mothers. There were no statistically significant differences between baseline values for any of the substances studied.

GLUCOSE

Baseline glucose values were obtained from cord samples in the 3 hour group and from heelstick determination in the 12 hour group. The early group showed a median cord blood glucose of 119.3 ± 37 mg/ml (mean ± S.E.M.). This corresponds to data obtained by King and others (18) who reported a cord value of 129.0 ± 12.0 mg/100 ml for infants of diabetic mothers. The baseline value for the later group was 48.4 ± 5.9 mg/100 ml (mean ± S.E.M.). This value also correlates with available data for IDM's at this age.

The blood glucose levels for the early group showed no significant rise in response to the intravenous alanine infusion. Although blood glucose levels were not known at the exact time of infusion, there was no significant rise in plasma glucose levels from 15 minutes after infusion (36 ± 8.0 mg/100 ml) to the completion of the study at 1 hour after infusion (37.6 ± 9.4 mg/100 ml) (p greater than .05). These results are similar to those obtained by Williams et al (29) in normal infants given 89 mg/kg boluses of l-alanine.

They also agree with the results obtained in normal infants given 125 mg/kg infusions in Mestyan's work (33).

ALANINE

Significant elevations in plasma alanine levels were noted following infusion in both early and late groups. In the 3 hour group, the initial concentration was 277.2 ± 21.9 micro M/L. This compares with normal adult levels and normal newborn levels of 254 ± 22 micro M/L as reported by Haymond et al (32). The 12 hour group had baseline levels of 383.9 ± 51.2 micro M/L. This compared to a level of 1033 ± 133 micro M/L noted by De Lamater et al (29) in normal newborns at 12 hours of age.

The 3 hour group showed a significant (p less than .005) rise in blood alanine levels at 15 minutes post infusion. A peak of 1117.3 ± 452 micro M/L was reached at 15 minutes with a reduction to 634.3 ± 97 micro M/L at the end of the test (1 hour). There was not a significant difference between the mean baseline concentration and the final concentration.

The 12 hour group showed a similar rise in alanine levels reaching a mean peak of 1912 ± 184 micro M/L at 30 minutes. This represented a mean increase of 1528 ± 150 micro M/L. This peak value fell to a mean of 986.1 ± 79 micro M/L at the end of the study, a significant (p less than .001) elevation above the starting concentration.

GLUCAGON

Although elevations of serum glucagon levels were noted in both the early and late groups, the rise was not significant in the 12 hour group. The 3 hour group had mean baseline (cord) glucagon levels of 233.1 ± 100 pg/ml. The mean baseline value for the 12 hour group was 155 ± 33 pg/ml. These values correlate well with those observed in normal (29, 31) and small for gestational age infants (33).

INSULIN

Insulin values fell over the course of the study in both groups from the initial concentrations to those obtained at the end (1 hour later). Unfortunately, due to large standard errors, these differences were not statistically significant.

In the early group, the mean baseline insulin concentration was 151.5 ± 128.0 micro U/ml. This concentration decreased to 30.67 ± 2.33 micro U/ml by the end of the study. In the later group, the mean baseline concentration was 66.6 ± 21.4 micro U/ml, falling to a final concentration of 22.2 ± 4.7 micro U/ml by the end of the period. Both sets of values are appreciably higher than those obtained in normal and small for gestational age infants (31), and while the difference approches but does not attain statistical significance, the 12 hour group had a lower mean baseline insulin concentration (mean less than half) than the 3 hour group.

DISCUSSION

In order to adequately interpret the data presented and to make some conclusions about the physiology involved, it is worth reviewing some of the known interactions between alanine and the major glucogenic hormones.

The primacy of alanine in the glucogenic pathway has been alluded to earlier. After the work of Van Slyke and Meyer, London and his co-workers measured the arterio-venous differences in levels of aminos acids in human forearms. They found a uniformly negative difference in the levels of 11 of 17 studied amino acids. Of these amino acids, alanine was released from these tissues in the highest concentration, in some instances accounting for 30% of the amino acid content (37). This work has subsequently been supported by the findings of other investigators working with subjects in different states of fasting (38, 39, 20).

The significance of the discovery that alanine is released in these higher concentrations is magnified by the fact that alanine comprises no more than 10% of the total amino acid content in skeletal and cardiac muscle (20). This predominance of alanine as the major source of nitrogen in the intracellular pool has led investigators to speculate that there must be another source of derived alanine in these tissues. Felig has suggested that the source of alanine is pyruvate (38), which is transaminated in the muscle to create alanine. This observation is supported by the direct correlation between

levels of circulating alanine and circulating pyruvate (40), a relationship which is not found in the other circulating amino acids of man. Other conditions of hyperpyruvatemia such as muscular exercise (40) and inborn enzymatic defects (20,41) also demonstrate a commensurate hyperalaninemia. The possibility exists that the alanine which is released is not synthesized solely from pyruvate, but that the alanine might in fact be formed from the breakdown of other types of amino acids which are then converted to alanine in the muscle. This hypothesis has the advantage of explaining a recycling of various carbon skeletons back into the glycolytic pathway. Further evidence in support of this theory is offered by the observation that many tissues use alanine as a means of disposing of nitrogen from other amino acids (42).

The liver also appears to be important for the recycling of alanine in the glycolytic cycle. Studies on hepatic perfusion in dogs and man have shown that alanine is preferentially extracted from the blood stream in much higher levels than other amino acids (43, 44, 45). As was seen in the pattern of amino acid release from muscle, splanchnic uptake of amino acids shows alanine consumption to be the highest, accounting for nearly 50% of the total amount of amino acid extracted from the blood.

Perhaps one of the last links in this pathway involved establishing that the alanine was in fact incorporated in the glucose molecule. This was done in postabsorptive man and

showed that alanine was incorporated into glucose, with the optimal recovery of labeled product at between 30 and 60 minutes after infusion (20, 46). Additionally, studies in which alanine was given via catheter to a perfused liver have shown alanine induced the highest rise in glucose of any amino acid used (47).

This pathway from skeletal muscle to liver and back to skeletal muscle is subject to the usual regulatory control mechanisms which have long been known to control glucose homeostasis. Glucagon is known to be a stimulant to increased gluconeogenesis (48). One would expect, therefore, that glucagon would cause an increased production of glucose from alanine through gluconeogenesis. And, in fact, studies using labeled alanine have shown that incorporation into glucose increased in the presence of glucagon (49, 50). This stimulation of glucagon secretion has been shown to be inhibited by secretin in studies of dogs (51). The mechanism, while not precisely known, does not appear to involve the usual feedback by insulin. In addition, in the presence of glucagon, the liver shows a preferential uptake of alanine. This increase in uptake is most probably due to conversion of alanine to glucose rather than to increased cellular uptake per se (52). Studies in intact human subjects have demonstrated similar findings to those quoted above for liver perfusion tests (53). In addition, subjects with acute hepatitis and abnormal hepatic glucogeogenic mechanisms show

a decreased response to glucagon with respect to alanine (54). Glucagon also seems to be active at the other end of the cycle, causing an increase in extrahepatic metabolism of alanine as well (20).

The relationship between alanine and glucagon appears to work both ways. That is, studies have shown that increase alanine results in a concomittant increase in glucagon (55). This effect is essentially abolished by states in which there is increased glucose available, such as obesity (20), but is enhanced in conditions in which glucose availability is decreased, such as fasting. A direct linear relationship between alanine and glucose and glucagon has been demonstrated (56).

The relationship between insulin and amino acid levels has been known for some time (57). Study of various models has led to the elaboration of the scheme of insulin effect which seems to be enhancement of amino acid uptake by muscles and subsequent incorporation into muscle protein (20). These early, yet non-specific studies suggested to some investigators that the effect of insulin was via hypoaminoacidemia (58). Unfortunately, however, subsequent studies of the effects of insulin on individual amino acids has shown this is not to be a likely mechanism. In studies of specific amino acids, both in man and animals, the reduction in concentration following administration of exogenous insulin or stimulation of endogenous insulin via glucose administration is related to branched chain amino acids primarily (valine, leucine, and

isoleucine) with alanine being the only amino acid which does not show a consistent decline following these tests (20). In fact, some studies have shown a rise in alanine concentration following insulin increase (20). It seems likely from subsequent studies that insulin causes an increase in peripheral alanine formation. This increase is most likely due to the transport of glucose into the cells under the influence of insulin. The flux of glucose results in a surplus of pyruvate which may be subsequently transaminated to form glucose (alanine) (21, 59). Therefore, the observed levels of alanine following insulin are the results of a counterbalance of increased production and increased absorption. If, therefore, insulin does not cause a decrease in available alanine, and if, in fact, alanine is the source of glucose in the gluconeogenic pathways, the control of gluconeogenesis from alanine by insulin must reside in the hepatic portion of the cycle.

In studies on intact human subject, Felig and Wahren (60) found that a hyperinsulinemic state, with the subsequent fall in hepatic glucose output, caused a decrease in hepatic uptake of alanine of 30-60%. Other amino acids were not similarly affected. These studies in intact subjects are supported by other work with perfused livers which has demonstrated that the amount of labeled alanine in glucose produced insulin. Thus the liver seems to differ from other tissue in its response to insulin. Unlike the protein incorporation seen in muscle in response to insulin, the liver

seems to be unaffected by insulin with respect to protein incorporation (20).

In studies of perfused rat livers, Sladek and Snarr showed that there is a significant inhibition of gluconeogenesis in the presence of insulin. This inhibition could be expressed in terms of a linear equation (Rate of gluconeogenesis (micro m/hr/g wet wt) = 15.93 - 0.0084 (Insulin, micro U/ml)). This inhibition was felt to be a direct inhibition of gluconeogenesis rather than an increase in conversion of glycogen, since previous studies by Jefferson et al using labeled glucose had shown that insulin inhibits gluconeogenesis without increasing labeling of glycogen (61).

The relationship between alanine and insulin, like the one between alanine and glucagon, seems to work both ways. The relationship between alanine and insulin seems to differ in some respects from a simple glucose-insulin stimulation. In studies of dogs which had been fasted for 5 days, Buckman et al found that while glucogenic insulin secretion was markedly reduced in these animals, the insulin response to alanine was not affected. While it is possible that the alanine is acting through glucagon stimulation (leading to insulin secretion) the authors point out that arginine causes a rise in glucagon levels without a concurrent insulin increase (62). Thus, the precise mechanism of alanine induction of insulin secretion is still unclear.

The sequence of biological events which occur following delivery of an infant has been fairly well established.

Metabolically, the clamping of the umbilical cord represents a marked diminution in the infant's supply of glucose. This results in increased pancreatic secretion of glucagon. Shortly thereafter, adenylcyclase is activated in hepatic and adipose tissue causing a commensurate increase in glycogenolysis and lipolysis. Shortly thereafter, the enzymes phophoenolpyruvate kinase, glucose-6-phosphatase and transaminases including tyrosine transaminase (TAT) become activated to increase the rate of gluconeogenesis. The exact order of these events, particularly glycogenolysis vs gluconeogenesis is unknown in man. In studies on the rat, gluconeogenesis appears to preceed glycogenolysis.

Although this classic description of events has been accepted for some time, there has been some discussion lately concerning the extent and nature of the glycemic response to alanine in man. Hahn, in a NEJM editorial, considered that response seen in infants given oral alanine loads. He pointed out that the infant's caloric requirements during the course of the study in question far exceed the amount provided by the alanine given. Since a glycemic response was noted, however, he suggests that this must be a function of increased glycogenolysis and gluconeognesis brought about by elevated glucagon levels, with the implication that alanine alone could not account for the glucose rise on a mole for mole basis (63).

There is evidence to suggest that the role of alanine in stimulating glucose production can be that of an inducer rather

than of substrate. In an elegant study by Friedrichs and Schoner, the effects of alanine levels on renal gluconeogenesis were investigated. Unlike its function in liver tissue, alanine is a very poor substrate for gluconeogenesis in the kidney. In addition, the renal cortex has a very low level of glycogen, so glycogenolysis would interfere relatively little. Using this model, Friedrichs found that alanine acted to produce a glucose rise which was independent of substrate concentration (catalytic). From their observations, they concluded that alanine was acting to inhibit pyruvate kinase (64). Considering data from other studies, the authors concluded that a certain amount of pyruvate (cycling) occurs in the liver under the influence of pyruvate kinase. In order to achieve effective glucose production, pyruvate kinase must be inhibited. Alanine appears to do just that.

However, data from other sources indicates that the glycemic response can, in some case, be accounted for on a mole-for-mole basis as well. From data with rat liver slices, it was estimated that a threefold increase in plasma alanine concentration could result in a 172% increase in the rate of gluconeogenesis (65). Additional data accumulated from rat liver perfusion studies has confirmed that seen in the liver slice studies (61). The authors of the latter study were able to derive a linear relationship between the rate of gluconeogenesis and alanine concentration in the range of .45-4.0 mM. (Gluconeogenesis (micro m/hr/g liver) = 0.61 + 5.53 (Alanine, mM).

In light of these two conflicting theories, one is prompted to suppose that the infants studied in the series reviewed by Hahn were in some sort of homeostatic balance prior to the alanine administration. Though it is unclear what mechanisms were supplying these infants with glucose at the time, the chances are that it was combined lipolysis and glycogenolysis. The alanine which was administered, therefore, was not needed to provide the infants with all the calories they needed to maintain normoglycemia, and they were able to show a glycemic response.

In the present study, two different responses to intravenous alanine administration were obtained. In the early group (3 hours) there was no significant glycemic response to alanine (in fact, glucose levels continued to drop in one infant). In the later group (12 hours) there was a marked glycemic response to the alanine. Interpreting the data from the early group is difficult, largely because of the small sample size. The reason for the small size is twofold. First, it was technically more difficult to perform the study on the younger infants. Besides the logistics involved in preparing the necessary equipment and obtaining consent within 3 hours or so, these infants proved to be more edematous, and it was harder to obtain blood in the amounts needed. Secondly, the sample size was limited by us for ethical reasons. After the first few infants were studied, it seemed quite likely that there would be no significant glycemic response to the alanine.

In fact, some infants entered the study but became hypoglycemic and had to be fed prior to completion of the study. For this reason, it was decided to change the time of study to 12 hours of age. These limitations do not invalidate the data obtained. However, it is not possible to rely as heavily on it as one would like.

What can be determined from the early group are a few trends. First, the failure to show a glycemic response to alane. Second, a relative rise in glucagon levels from baseline to peak. Third, a similar peak in alanine levels which was a significant increase from baseline levels. And, finally, a hyperinsulinemic state which moderated over the course of observation.

The simplest way to interpret these data is to explain them on the basis of hyperinsulinemia. It could be suggested that the abundance of insulin, presumably of maternal origin, blunted the infant's response to alanine. One would postulate that the pancreas responded as expected in these infants with an increase in glucagon, but that a) the liver did not extract the infused alanine in the presence of a relative hyperinsulinemia, b) the glucagon failed to produce the expected gluconeogenic or glycogenolytic response in the liver.

Of these, the first seems to be the most likely. As was mentioned previously, in work with perfused livers, it has been shown that insulin excess decreases the rate of hepatic alanine extraction (66). One would expect in such a case to see a rapid rise in alanine levels after infusion,

reaching a relatively high peak concentration, followed by a gradual fall off or plateau. Although the data is scanty here, this is, in fact, what is seen. The peak levels are not significantly different from those of the later group, but there is a more gradual fall of alanine levels in these infants than that seen in the later group.

The second possibility, that is, a failure of hepatic function, might also be considered. It is known that cyclic AMP or glucagon, particularly in combination with adrenal steroids, can bring about a 10 fold increase in the activity in the gluconeogenic enzymes PEPK and TAT as early as 20 weeks of gestation (67). One would assume, therefore, that term infants, as studied here, would be capable of converting substrate into glucose. The elevated levels of adrenal steroids found in IDM's (34) would lead one to suspect an acceleration in this pathway in fact.

Another possible cause for impaired gluconeogenesis, or more importantly, glycogenolysis, is depletion of hepatic reserves. In their studies on neonatal hypoglycemia, Cornblath et al concluded that the decreased response to glucagon administration in these infants prior to the development of symptoms indicated diminished hepatic glycogen reserves. After administration of parenteral glucose and steroids or ACTH, a normal response to glucagon was obtained. (68) Hahn, in his editorial, suggests that a condition of maximal glucose production might exist in some newborns

immediately after delivery. Presumably this increased rate might be related to an elevated "glucostat" set during a hyperglycemic pregnancy. If this were the case, administration of alanine, which would normally induce the production of glucose would not have any further effect on serum glucose levels. In infants in whom glucose production from glycogenolysis was not maximal, the alanine would presumably act in its usual way and a glycemic response would occur (63). Such a theory, however, must explain the observations of Williams et al that infants who show a glycemic response to alanine on the first day do not show such a response on the second day. The authors explain this discrepancy on the basis of a higher glucose level, which acts in some way to shut off a further glycemic response.

The second (later) group of infants studied confounds the results from the earlier group in many ways. As mentioned previously, these newborns showed a significant glycemic response to the infused alanine during the course of the study. The rise in glucose was associated with a rise (although not statistically significant) in glucagon, which began shortly after injection of the alanine. Alanine levels in these infants rose to higher levels than those seen in the earlier group, and the final levels of alanine were elevated above the initial concentrations as were those seen earlier. Insulin levels in these infants were elevated above normal, but not as much as were those in the earlier infants.

One would like to attribute the difference in responses to the lower insulin levels in this group. One could postulate in that event that the first group failed to respond merely because of a hyperinsulinemia. However, as appealing as this theory is, there are several inconsistencies with it.

First, the insulin levels in the later group are still above normal, and, although they are lower than the early group, the difference is not great. In their studies with perfused rat livers, Sladek and Snarr found that a significant inhibition of gluconeogenesis could be produced by as little insulin as 10 micro U/ml rising to a maximum inhibition in the range of 500 micro U/ml (61).

Secondly, were the differences due to hyperinsulinemia, one might expect that the peak alanine levels seen in the second group would be lower than those seen in the early group. Presumably, with less insulin to interfere a higher rate of gluconeogenesis would exist, and the alanine would be used more rapidly. The final concentration (60 minutes) might be expected to be lower than in the early group as well, since presumably more alanine would then have been converted to glucose. As can be seen from the data, this is not the case.

A more intriguing approach concern glycogen metabolism in these infants. It is well known that in the immediate post partum period, the infant mobilizes glycogen stores at a rapid rate. In addition, fat in subcutaneous tissue

is also used. This increase in glycogenolysis is known to start prior to delivery and continue at a high rate through the first few hours of life, until exogenous sources become available. IDM's have been found to have higher glycogen stores at birth as well as increased rates of glycerol release from these tissues after birth (69). It is felt that the neonate uses glycogen stores preferentially, reducing the amount of FFA which is released into the circulation (70). A fall in the levels of ATP in these tissues is also noted during this time (69).

From these observations, one would expect that the infants in the first group were experiencing a high, and perhaps maximal glycogen turnover at the time of the study. If alanine were acting through increased glucagon and a resultant glycogenolysis, one would expect to see little if any response to the infusion of alanine, since rates were maximal at the outset. In the later group, which had been fed prior to the study, one might expect to find a lower rate of glycogenolysis at the beginning of the study. They would be able to increase the rate, and show a glycemic response.

Although earlier studies have shown that glycogen stores become depleted with time following delivery (71) in the present study, the infants in the later group were fed prior to the start of the alanine infusion. One might suggest that glycogen stores were either replenished or in the process of being replenished and were, therefore, responsive to alanine-induced, glucagon-mediated glycogenolysis. One

would not have to invoke increased gluconeogenesis to account for this response, thus circumventing the problems associated with the hyperinsulinemia which still existed at the later time. Nor would one expect to see significant changes in the alanine-disappearance rate, since the alanine would not be used up in the glycemic response. All of this presupposes that the infant's glycogen stores were sufficient at 12 hours of age after feeding to support the required substrate for the glycemic response. We have no way of knowing that this is the case, but we do know that insulin increases the rate of glycogen formation following feeding (69). Thus, one might postulate that the same condition which resulted in a failure of the early group to respond to alanine infusion (hyperinsulinemia) brought about the increased glycogen stores which allowed the later group to respond.

The hypothesis proposed above conflicts somewhat with observations of Pildes et al (72). They found that infants primed with dextrose prior to glucagon infusion did not respond to glucagon as did normal infants. They speculated that this might be due to antagonizing effects of glucagon and insulin in the liver. Were this the case in the present study, one would expect much different results than those obtained. However, there are significant differences between that study and this. In the Pildes study, the infants who did not respond were primed with intravenous dextrose as a 10% solution. This infusion was not stopped until 30

minutes prior to the study. Their controls, however, were fasted for 3-6 hours prior to study, depending on the age of the infant. In our study, the older group was fed orally at intervals of 3 hours, which is our usual feeding regimen for such infants. These infants were then fasted for four hours prior to study. In this respect, therefore, the older infants in our study were more similar to the control infants in the Pildes study, which did respond to glucagon (72).

Evidence accumulated from the present study also stands in opposition to that presented by Bloom and Johnston (73). They studied fifteen infants of diabetic mothers and compared them with normal infants and small for dates infants. They showed that at two hours of age, infants of diabetic mothers had a significantly smaller rise in glucagon levels than did the other groups. Although our early group was very small, results obtained from them at 3 hours showed them to have a greater increase in glucagon levels than the later group when challenged with alanine. Infants in the later group showed glucagon elevations which were greater than those observed in Bloom's normal controls. These discrepancies can be explained in terms of a biofeedback system. The early group in our study seemed to produce glucagon adequately in response to alanine, a known glucagon stimulant. However, if one supposes that these infants, as previously suggested, were unable to a) clear alanine via gluconeogenesis or b) produce glucose, and thereby "shut off" glucagon production via glycogenolysis,

one would expect to see the high levels obtained in our study. One should also point out that all of the infants studied in Bloom's group were infants of Class B or worse diabetics. One would suppose that insulin levels in these infants were significantly higher than those obtained from our study of primarily Class A diabetics. Since the number of infants studied in our protocol was very small, one is reluctant to ascribe too much significance to this difference in data.

One might argue that the difference noted in the two groups of infants demonstrates a relative phosphoenolpyruvate carboxykinase deficiency early in life with a subsequent appearance by 12 hours of age which enables the older infants to respond. It has been shown in experiments with rats (20) that the level of PEP carboxykinase increases dramatically from the time of birth until it reaches a plateau at about 8 days of age. Although no such data exists for man, it could be argued that a similar process at work here prevented the 3 hour group from responding, while the later group did.

Several points mitigate against this explanation. First, we have evidence from studies of normal infants (31) that they are capable of responding to alanine infusion at an early age. Admittedly, however, many of the infants in that study did not respond until 36 hours. Secondly, the alanine disappearance curves, while not a precise measure of

utilization, would seem to indicate that there was, if anything, more substrate utilization in the 3 hour group. Finally, as pointed out earlier, the magnitude of the glycemic response seen exceeds that which can be accounted for by direct conversion of alanine to glucose, an indication that gluconeogenesis alone is not responsible for the glycemic response.

All of this notwithstanding, our data does not rule out the possibility of PEP carboxykinase deficiency in these infants. The fact that such a state seems to be more or less universal in newborns suggests to us that there is more involved in the hypoglycemia of diabetic infants. Further and more specific studies must be undertaken to determine this more precise information.

SPECULATION

One is now left with the difficult problem of putting all of these assorted bits and pieces of information into some sort of unified concept. We would not pretend to be able to elaborate a complete pathway on the basis of our current data; however, it is tempting to speculate as to the mechanisms involved. In that spirit, one can construct the following picture.

Alanine's mode of action is via two major pathways: gluconeogenic and glycogenolytic. The gluconeogenic pathway may be subdivided into substrate mode and catalyst mode action. The substrate pathway operated through the conversion of alanine into glucose using the normal gluconeogenic pathways. The catalytic process seems to work through an inhibition of pyruvate kinase. The inhibition of pyruvate kinase causes an increase in glucose production through a re-channeling of phosphoenolpyruvate which proceeds up the gluconeogenic pathway to glucose. This inhibition of pyruvate kinase was overcome by fructose-1, 6-diphosphate in studies on the rat kidney (21). There is no evidence as yet what the effects of either insulin or glucagon are on this process. However, the authors of that study indicated that the pathway might be different from the known process which involves cyclic AMP.

The substrate mode of action seems to relate to levels of both glucagon and insulin. Although it has been suggested that insulin has a blocking action on gluconeogenesis

independent from glucagon levels, (61) there is more evidence to suggest that the rate of gluconeogenesis is a function of the molar ratios of glucagon to insulin (72). Thus one might expect that in patients such IDM's, the high insulin level could be overcome by a sufficiently high glucagon level, and that the insulin level by itself is less important in determining whether or not gluconeogenesis is to proceed.

The glycogenolytic mechanisms referred to are probably mediated by glucagon. Alanine is known to be a potent stimulus to glucagon secretion (76). In the dog, this stimulation can be effectively blocked by the administration of secretin (75). Glucagon may be acting through stimulation of cAMP, activating phosphorylase, and deactivating glucagon synthetase. Glucagon stimulated glycogenolysis may be blocked by insulin, which is also stimulated by alanine. It has been suggested that insulin opposes the action of glucagon in this regard by means of a second messenger, opposing the action of cAMP (72). The levels of cAMP-stimulated phosphorylase and cAMP-inhibited glycogen synthetase may be related to environmental factors, and may constitute a "glucostat" as postulated by one author. One might suppose that membrane phenomena might alter the sensitivity of this sytem to various levels of glucagon in order to maintain homeostasis. Insulin has also been shown to stimulate glycogen synthetase.

Glucose produced from either or both of these systems is known to stimulate insulin production. It has also been

shown that this stimulation is blocked by starvation, whereas alanine stimulation of insulin is not (62). Glucose is metabolized to form pyruvate in some instances, and pyruvate may then be converted into alanine in the muscle for re-entry into this system. Glucose can also act to interfere with the alanine-stimulated secretion of glucagon.

In light of these facts and the evidence presented in the body of this paper, one can postulate that in the three hour group, insulin levels were such that the alanine-mediated glucagon secretion was not sufficient to overcome the effects of a relative hyperinsulinemia and cause a resulting gluconeogenesis. However, in light of the concentration of alanine infused and the demands of a newborn infant at this stage, this must be considered a less likely explanation. As Hahn points out in a criticism of a similar study (63), the amount of alanine given in this study cannot explain a rise in blood glucose if suggested on a mole-for-mole basis. It is possible, however, that the effect of alanine was catalytic in nature as mentioned earlier. This would pre-suppose, however, that there was an abundance of other substrate present at the time from which to make glucose. Given the period of fasting after birth and the demands of a stressful environment, it is unlikely that there is such a surplus present.

Rather, it seems more likely that glycogenolysis is proceeding at a rapid rate at this time the baby's life. With production of peripheral glucose proceeding at near maximal rates without any stimulus, it is unlikely that

the presence of alanine could cause a detectable rise in peripheral glucose. One would imagine that any extra glucose production from such a stimulus would be undetectable by our methods.

By 12 hours of age, the insulin levels had moderated somewhat. In addition, since the infants had been fed, one can postulate that glycogen was being formed from exogenous glucose, and that glycogenolysis was being inhibited by decreased glucagon levels and insulin inhibition of cAMP. Following administration of alanine to this group, inhibition of pyruvate kinase can be postulated, as well as stimulation of glucagon. In the presence of lower levels of insulin, the glucagon secretion was sufficient to make the glucagoneogenic system receptive to substrate.

However, in light of what has been previously stated concerning the amount of alanine given, its inadequacy as substrate, and the unlikely presence of excess substrate of other kinds following a four hour fast, we must favor a glycogenolytic mechanism to explain the rise in glucose seen in the later group of infants. Perhaps following feeding partially repleted glycogen stores were being used at submaximal rates. Following the infusion of the alanine, a rise in glucagon levels occurred. This added glucagon stimulation resulted in a return to maximal glycogenolysis and a resultant glycemic response.

APPENDIX

Table 1 summarizes the results of serial glucose determinations at 3 and 12 hours of age. These data are presented graphically as absolute (Figure 1) and incremental values (Figure 2). As can be seen, there is no significant increase in glucose values after infusion in the 3 hour group while the 12 hour group showed a marked elevation.

Table 1

GLUCOSE (mg/dl)

3 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	119.33333	64.66323	37.33333
15	36.00000	13.89244	8.02081
30	34.66667	19.85783	11.46492
60	37.66667	16.28906	9.40449

3 HOURS (Change)

TIME	MEAN	STD. DEV.	STD. ERR.
0	0.00000	0.00000	0.00000
15	-83.33333	76.56588	44.20533
30	-84.66667	82.52474	47.64568
60	-81.66667	79.05273	45.64111

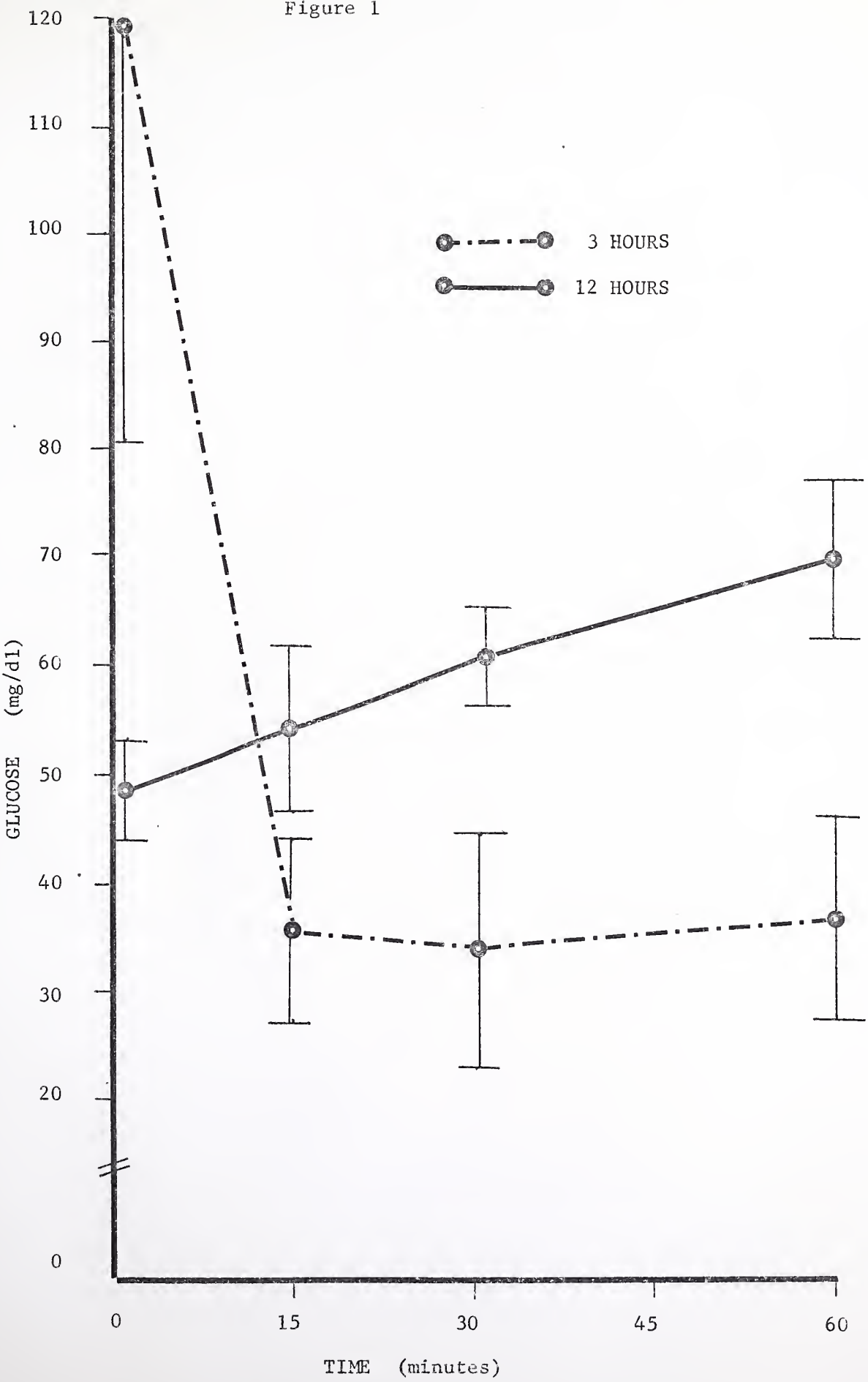
12 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	48.82000	13.24092	5.92152
15	54.04000	16.39857	7.33366
30	61.72000	13.33480	5.96351
60	70.84000	16.98906	7.59774

12 HOURS (Change)

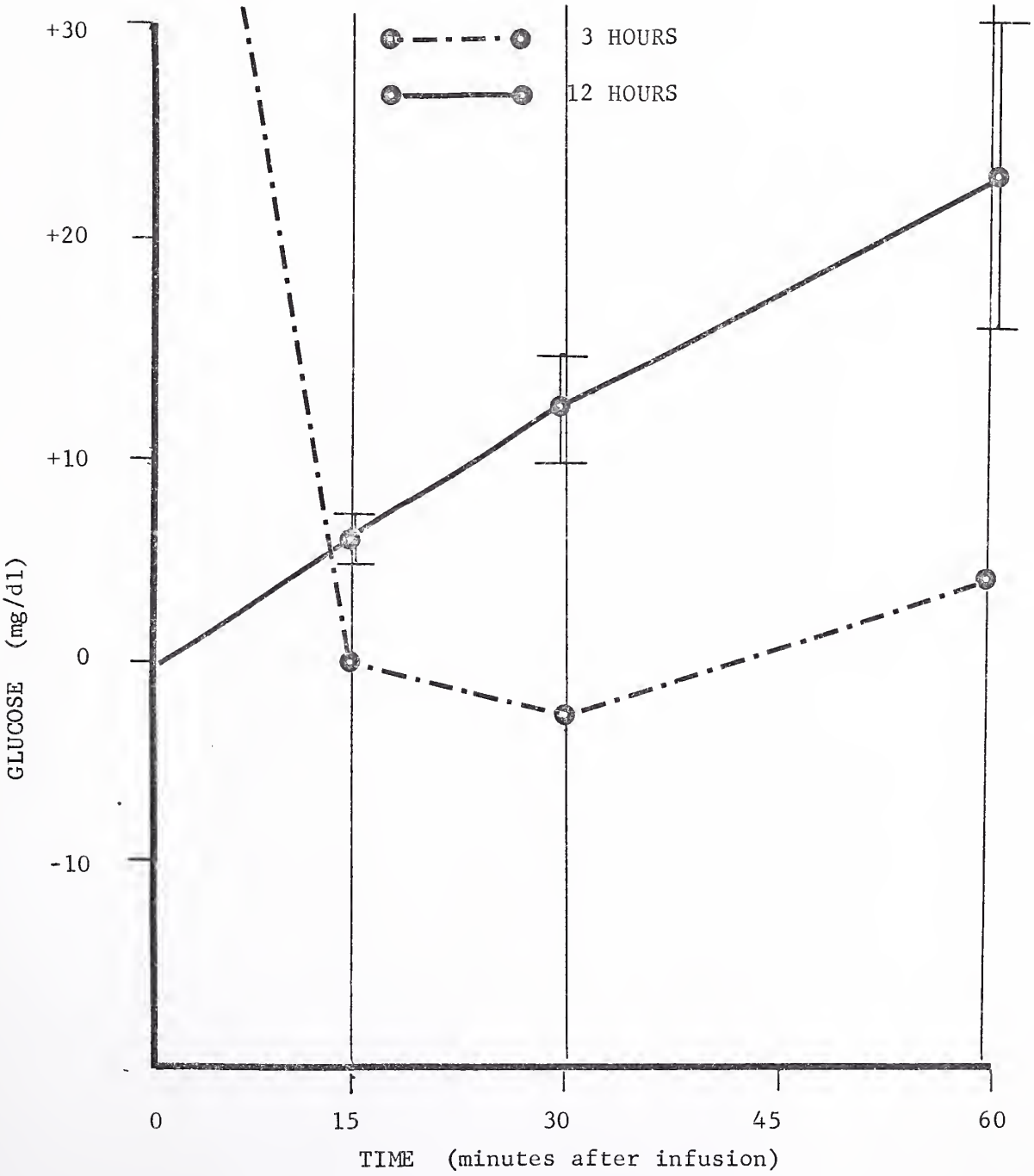
TIME	MEAN	STD. DEV.	STD. ERR.
0	0.86000	1.92303	0.86000
15	6.08000	3.52945	1.57842
30	12.90000	5.98916	2.67843
60	22.02000	16.05372	7.17944

Figure 1



GLUCOSE (Absolute)

Figure 2



GLUCOSE (Change)

Table 2 summarizes the data from alanine determinations during the study. Both groups show significant increases (Figures 3 and 4) in alanine levels within 15 minutes after infusion. Although the 12 hour group showed higher serum levels after infusion, the difference was not significant.

Table 2

ALANINE (μM/ L)

3 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	277.16667	38.00110	21.93994
15	1117.33333	783.17963	452.16897
30	1060.50000	67.86199	39.18014
60	634.33333	169.10450	97.63253

3 HOURS (Change)

TIME	MEAN	STD. DEV.	STD. ERR.
0	0.00000	0.00000	0.00000
15	840.16667	764.25165	441.24090
30	783.33333	89.78354	51.83655
60	357.16667	149.38736	86.24883

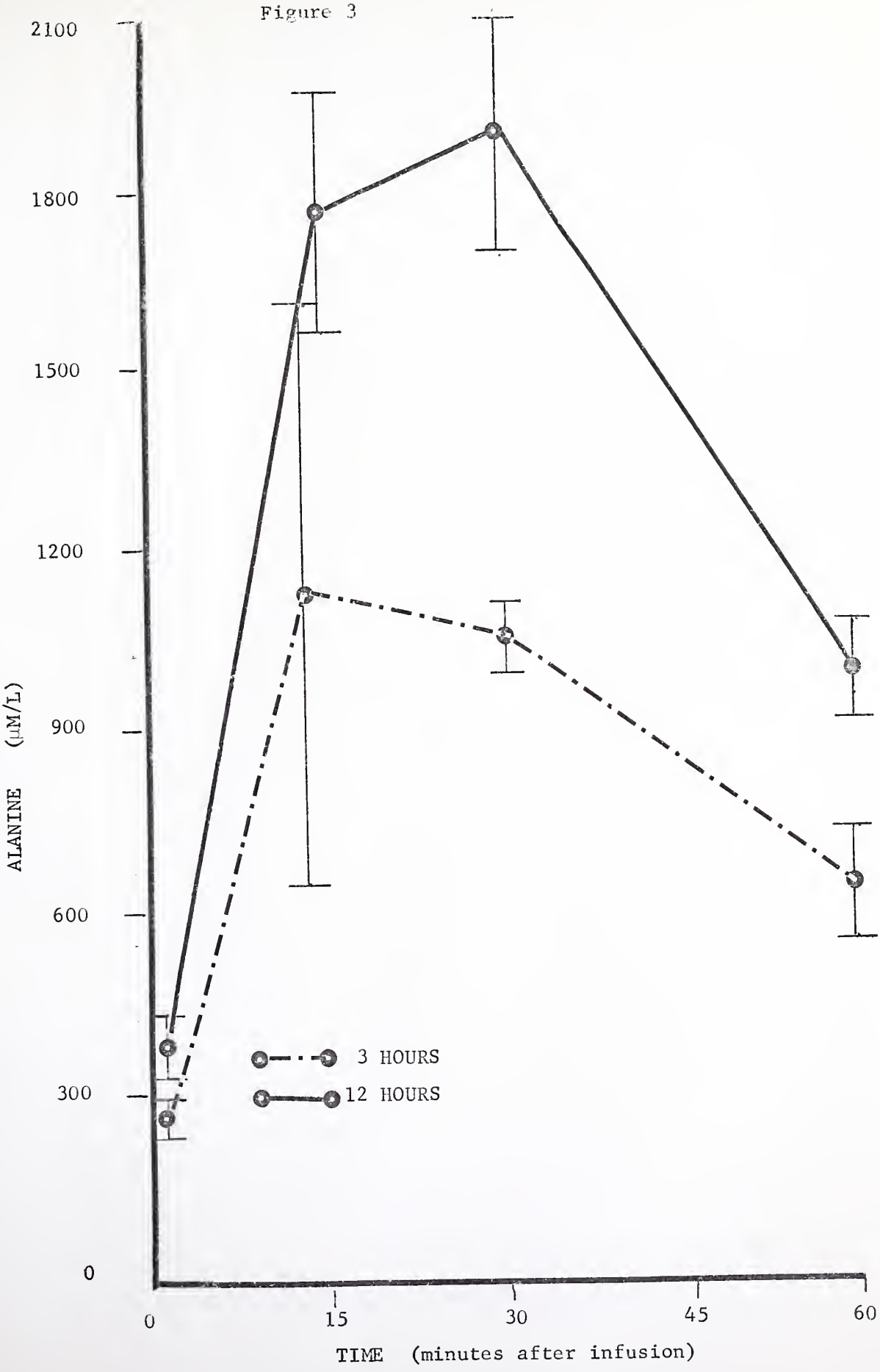
12 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	383.90000	114.47401	51.19434
15	1792.30000	356.72496	159.53225
30	1912.60000	412.09501	184.29449
60	986.10000	177.40011	79.33574

12 HOURS (Change)

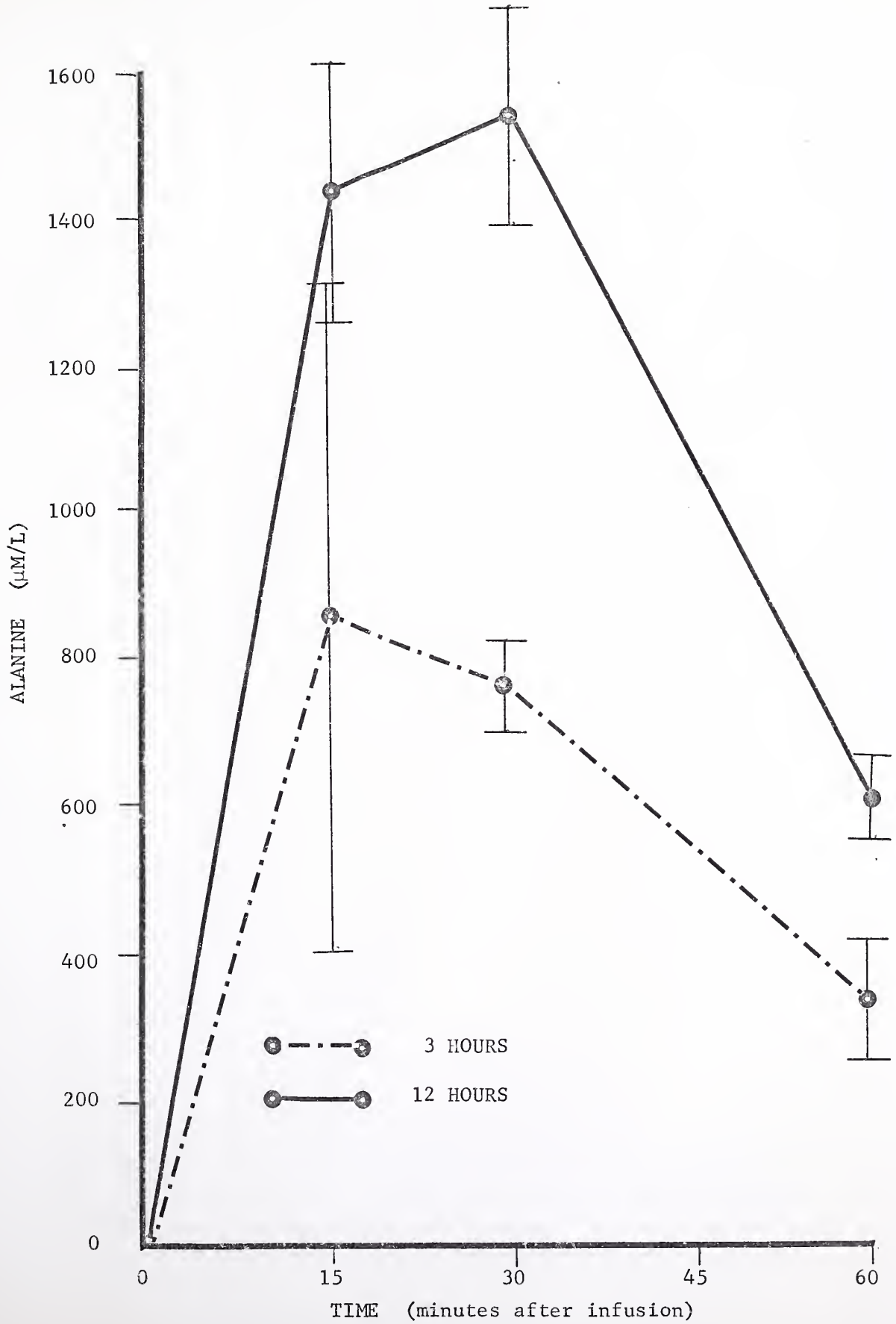
TIME	MEAN	STD. DEV.	STD. ERR.
0	0.00000	0.00000	0.00000
15	1408.40000	442.16377	197.74165
30	1528.70000	336.68710	150.57105
60	602.20000	103.00279	46.06425

Figure 3



ALANINE (Absolute)

Figure 4



ALANINE (Change)

Table 3 shows the glucagon values and increments above baseline following infusion. As can be seen from the graphs (Figures 5 and 6) the alanine produced a significant glucagon response in both groups of infants. The 3 hour group continues to increase throughout the study while the 12 hour group shows a decrease after 30 minutes.

Table 3

GLUCAGON (pg/ml)

3 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	233.13333	174.72107	100.87526
15	521.66667	105.39608	60.85045
30	441.66667	140.56434	81.15486
60	651.66667	359.03807	207.29072

3 HOURS (Change)

TIME	MEAN	STD. DEV.	STD. ERR.
0	0.00000	0.00000	0.00000
15	288.53333	174.94414	101.00405
30	208.53333	77.83607	44.93867
60	418.53333	211.89963	122.34031

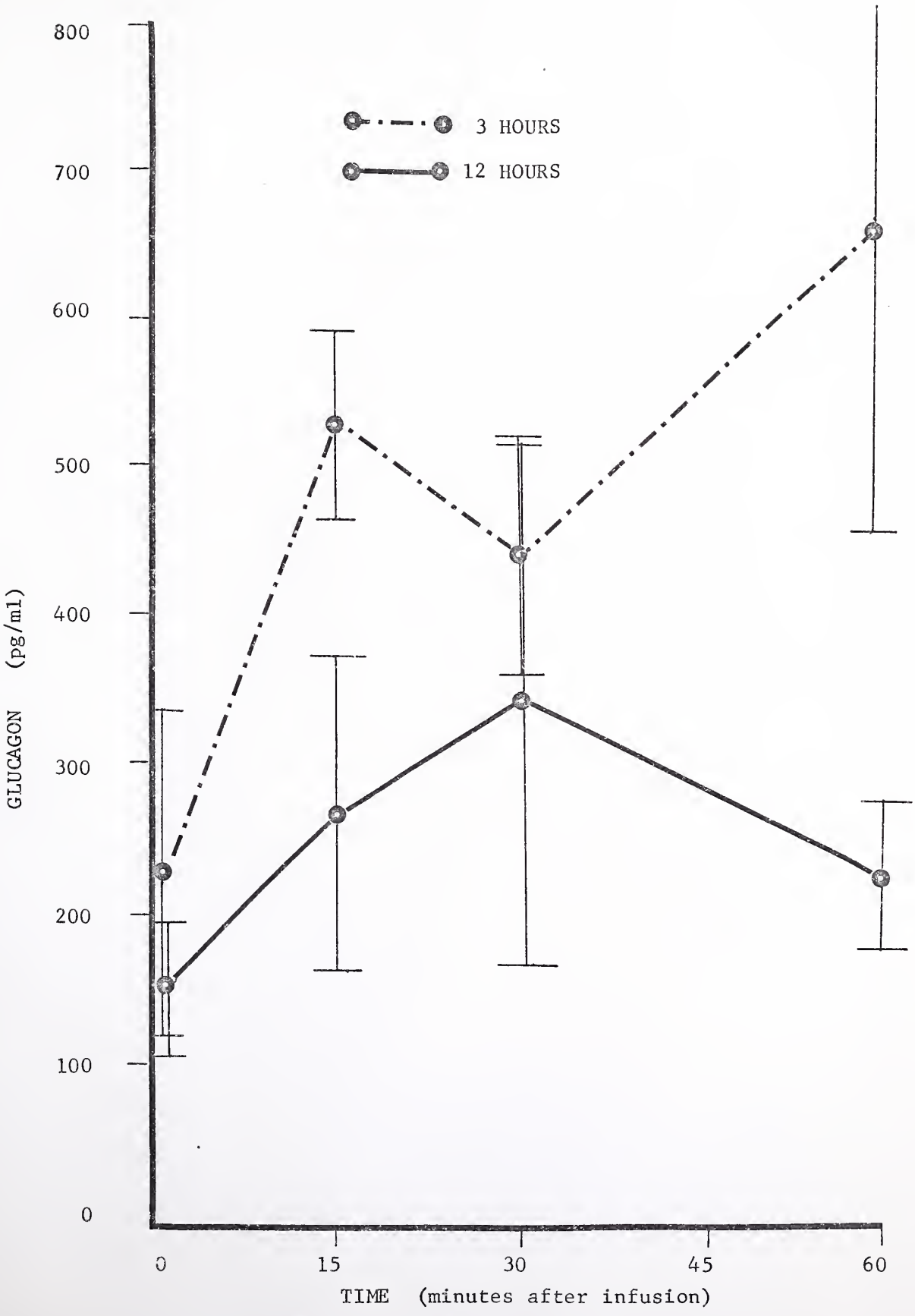
12 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	155.60000	75.96825	33.97403
15	268.00000	222.48174	99.49686
30	332.30000	380.18689	170.02475
60	226.80000	107.80747	48.21297

12 HOURS (Change)

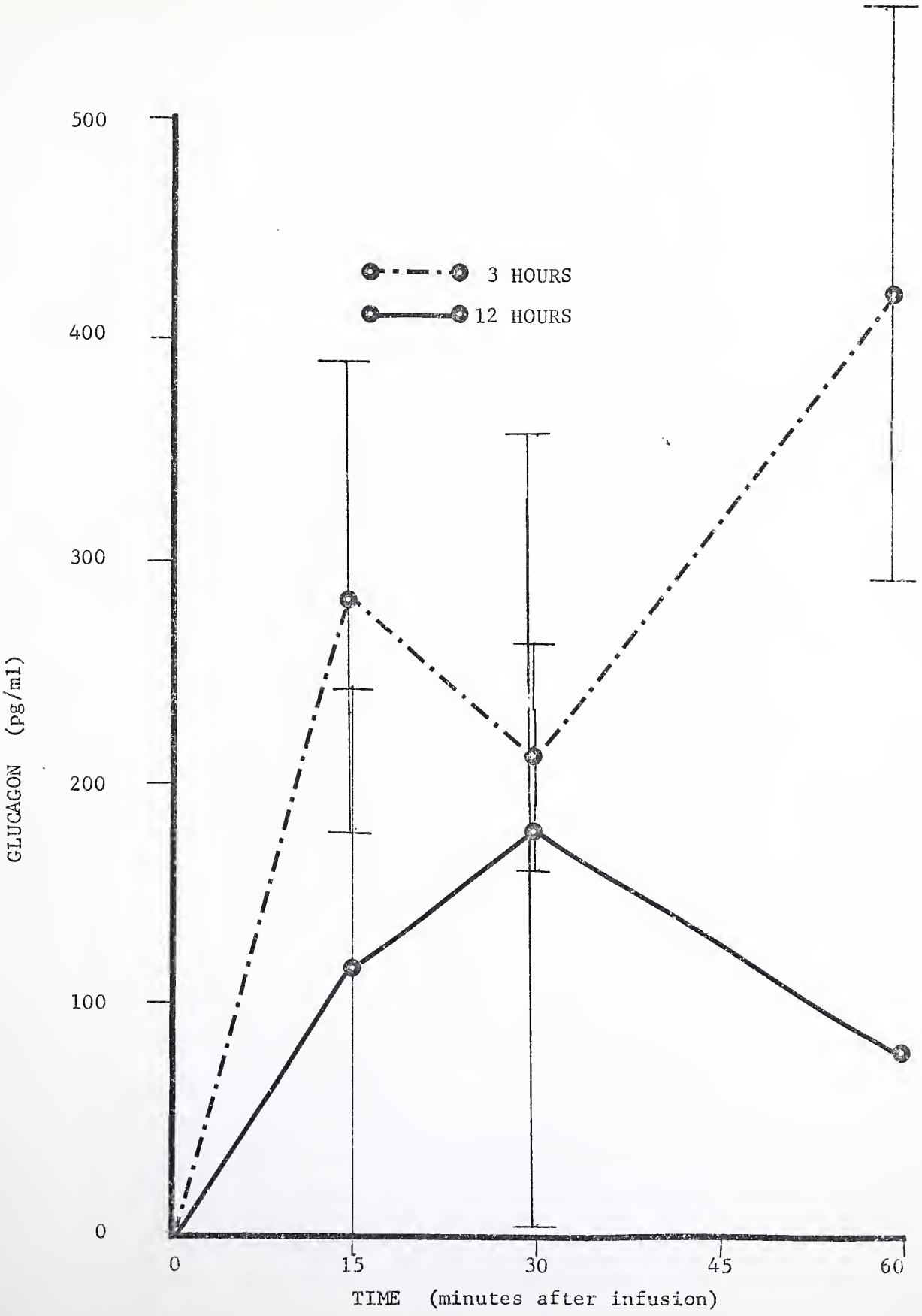
TIME	MEAN	STD. DEV.	STD. ERR.
0	0.00000	0.00000	0.00000
15	112.40000	259.34061	115.98064
30	176.70000	398.10781	178.03922
60	71.20000	115.10897	51.47830

Figure 5



GLUCAGON (Absolute)

Figure 6



GLUCAGON (Change)

Table 4 tabulates the results of insulin assay from three points during the study. As the graph (Figure 7) illustrates, both groups showed a decline in blood insulin over the course of study. Although there is a large discrepancy between initial values in the two groups, because of large standard errors in the early group, these differences were not significant.

Table 4

INSULIN (μU/ml)

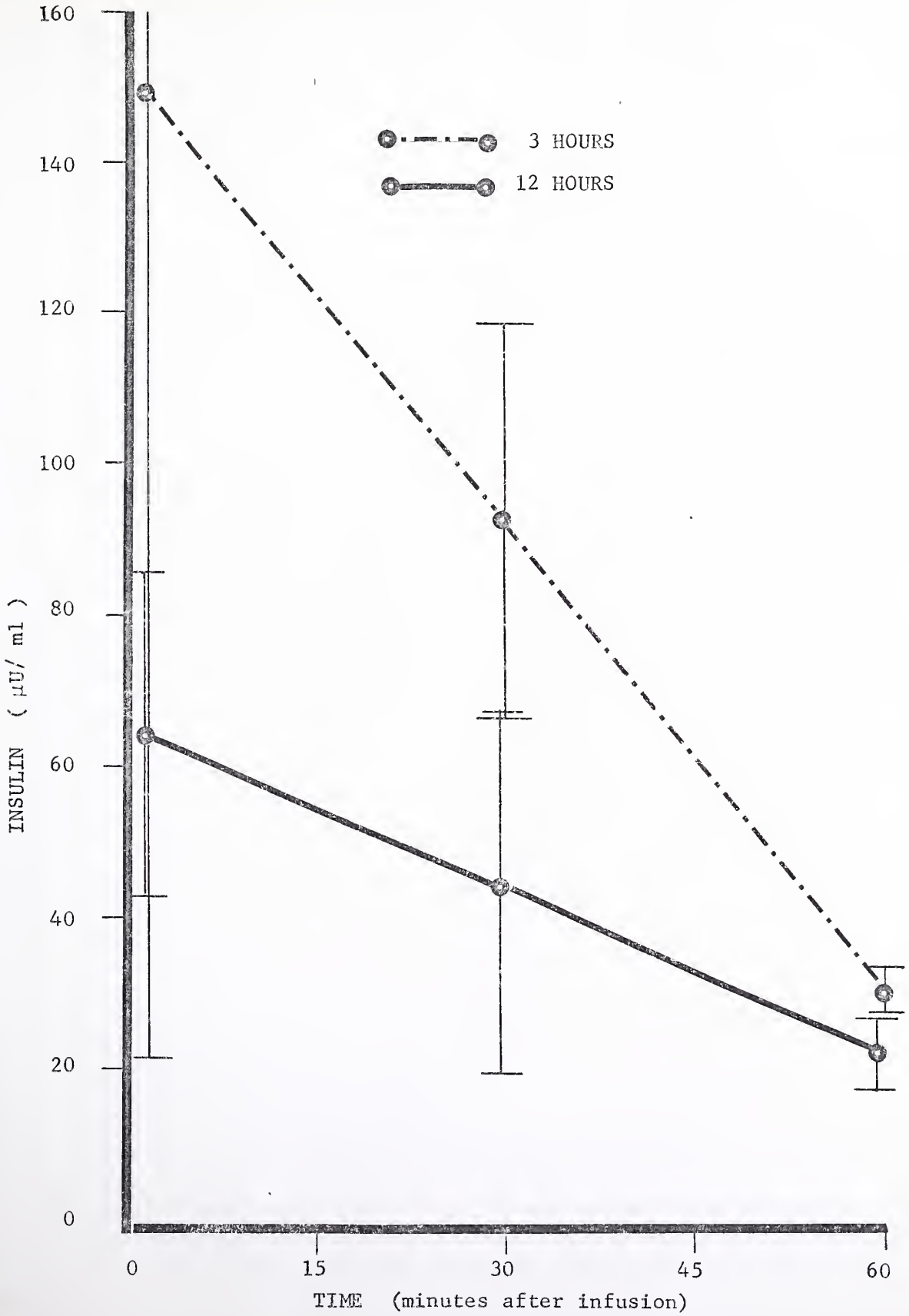
3 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	151.50000	221.70758	128.00293
30	92.33333	45.34681	26.18099
60	30.66667	4.04145	2.33333

12 HOURS (Absolute)

TIME	MEAN	STD. DEV.	STD. ERR.
0	66.65750	42.75651	21.37826
30	44.76400	51.91367	23.21650
60	22.16400	10.66775	4.77076

Figure 7



INSULIN (Absolute)

REFERENCES

1) Hartmann, A.F., J.C. Jaudon, 1937, Hypoglycemia. J. Pediatrics 11:1-36.

2) Oakley, W., 1953, Prognosis in diabetic pregnancy. Brit. Med. J., 1:1413.

3) Farquhar, J.W., T.E. Isles, 1968. Hypoglycemia in newborn infants of normal and diabetic mothers. South African Medical Journal 42:237.

4) Pildes, R., et al, 1967. The indidence of neonatal hypoglycemia -- a completed survey. J. Pediatrics 70:22.

5) Komrower, G.M., 1954. Blood sugar levels in babies born of diabetic mothers. Arch. Dis. Child. 29:28.

6) Farquhar, J.W., 1956. The significance of hypoglycemia in the newborn infant of the diabetic woman. Arch. Dis. Child. 31:203.

7) Beard, A. et al, 1971. Neonatal hypoglycemia -- a discussion. J. Pediatrics 79:314.

8) Hicks, S.P., 1953. Developmental brain metabolism. Arch Path 55:302.

9) Himwish, H.E., et al, 1941. Cerebral blood flow and brain metabolism during insulin hypoglycemia. Amer. J. Phsiol. 132:640.

10) Koivisto, M., Blanco-Sequeiros, M. and Krause, Ulf, 1972. Neonatal symptomatic and asymptomatic hypoglycemia: a follow-up study of 151 children. Devel. Med. Child. Neurol. 14:603.

11) Driscoll, S.G., K. Bernirischke, and G.W. Curtis, 1960. Neonatal deaths among infant of diabetic mothers: post mortem findings in ninety-five infants. Amer. J. Dis. Child. 100:818.

12) Cardell, B.S., 1953. Hypertrophy and hyperplasia of the pancreatic islets in newborn infants. J. Path. Bact. 66:335.

13) Osler, M., 1960. Body fat of newborn infants of diabetic mothers. Acta Endocrin. 34:277.

14) Miller, J.C., 1956. Offspring of diabetic and prediabetic mothers. Advances in Pediat. 8:137.

15) Chem, C.H., et al, 1965. The plasma free fatty acid composition and blood glucose of normal and diabetic pregnant women and of their newborns. Pediatrics. 36:843.

16) McCann, M.L., et al, 1966. Effects of fructose on hypoglycemia in infants of diabetic mothers. New Eng. J. Med. 275:1.

17) Baird, J.D., J.W. Farquhar, 1962. Insulin-secreting capacity in newborn infants of normal and diabetic women. Lancet 1:71.

18) King, K.C., et al, 1969. Infants of diabetic mothers: attenuated glucose uptake without hyperinsulinemia during continuous glucose infusion. Pediatrics 44:381.

19) Van Slyke, D.D., G.M. Meyer, 1913. The fate of protein digestion products in the body. V. The effects of feeding and fasting on the amino-acid content of the tissue. J. Biol. Chem. 16:231.

20) Felig, P., 1973. Progress in endocrinology and metabolism: The glucose-alanine cycle. Metabolism. 22:179.

21) Carlsten, A., et al, 1966. Amino acids and free fatty acids in plasma in diabetes. Acta Med. Scand. 179:361.

22) Wahren, J., et al, 1972. Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. J. Clin. Invest. 51:1870.

23) Felig, P. et al, 1970. Plasma amino acid levels in diabetic ketoacidosis. Diabetes 19:727.

24) Adam, P.A.J., 1971. Control of glucose metabolism in the human fetus and the newborn infant. Adv. Metab. Disord. 5:184.

25) Cahill, G.F., A. Zottu, A.S. Earle, 1957. In vivo effects of glucagon on hepatic glycogen phosphorylase and glucose-6-phosphatase. Endocrinology 60:265.

26) Exton, J.H., 1972. Gluconeogenesis Metabolism 21:945.

27) Sperling, M.A., et al, 1974. Spontaneous and amino acid stimulated glucagon secretion in the immediate postnatal period: Relation to glucose and insulin. J. Clin. Invest. 53:1159.

28) Wise, J.K., et al, 1973. Evidence of stimulation of glucagon secretion by alanine in the human fetus at term. J. Clin. Endocrinol. Metabl. 37:345.

29) DeLamater, P.V., et al, 1974. Plasma alanine: Relation to plasma glucose, glucagon, and insulin in the neonate. J. Pediatrics 85:702.

30) Gutberlet, R.L., et al, 1974. Alanine utilization in the newborn. Pediat. Res. 433:159.

31) Williams, P.R., et al, 1975. Effects of oral alanine feeding on blood glucose, plasma glucagon, and insulin concentrations in small-for-gestational age infants. New. Eng. J. Med. 292:612.

32) Haymond, M.W., I.E. Karl, A.S. Pagliara, 1974. Increased gluconeogenic substrates in the small-for-gestational age infant. New Eng. J. Med. 291:322.

33) Mestyan, J.K., K. Schultz, and M. Horvath, 1974. Comparative glycemic response to alanine in normal terms and small-for-gestational age infants. J. Pediatrics 85:275.

34) White, Priscilla, 1974. Diabetes mellitus in pregnancy. Clinics in Perinatology 1:331.

35) Farquhar, J.W., 1962. Maternal hyperglycemia and foetal hyperinsulinism in diabetic pregnancy. Postgrad. Med. J. 38:612.

36) Lowry, Oliver H. and Passeneau, Janet V., A Flexible System of Enzymatic Analysis. New York: Academic Press, Inc. 1972. Page 146.

37) London, D.R., T.H. Foley, and C.G. Webb, 1965. Evidence for the release of individual amino acids from the resting human forearm. Nature (London) 208:588.

38) Felig, P., T. Pozefsky, E. Marliss, and G.F. Cahill, Jr., 1970. Alanine: key role in gluconeogenesis. Science 167:1003.

39) Pozefsky, T., P. Felig, J. Tobin, J.S. Soeldner, and G.F. Cahill Jr., 1969. Amino acid balance across the tissue of the forearm in postabsorptive man: effects of insulin at two dose levels. J. Clin. Invest. 48:2273.

40) Felig, P., and J. Wahren, 1971. Amino acid metabolism in exercising man. J. Clin. Invest. 50:2703.

41) Lonsdale, D., W.R. Faulkner, J.W. Price, and R.R. Smeby, 1969. Intermittent cerebellar ataxia associated with hyperphyruvic acidemia, hyperalaninemia, and hyperalaninuria. Pediatrics 43:1025.

42) Coulson, R.A., and T. Hernandez, 1968. Amino acid catabolism in the intact rat. Am. J. Physiol. 215:741.

43) Felig, P., O.E. Owen, and J. Wahren, 1969. Amino acid metabolism during prolonged starvation. J. Clin. Invest. 48:584.

44) Onen, K.H., O.L. Wade, and J.D. Blainey, 1956. Amino acids in hepatic venous and arterial blood. Lancet 2:1075.

45) Carlsten, B., B. Hallgren, et al, 1967. Arterio-hepatic venous differences of free fatty acids and amino acids. Acta Med. Scand. 181:199.

46) Kressberg, R.A., A.M. Siegal, and W.C. Owen, 1972. Alanine and gluconeogenesis in man: effect of ethanol. J. Clin. Endocr. 34:876.

47) Ross, B.D., R. Hems, and H.A. Krebs, 1967. The rate of gluconeogenesis from various precursors in the perfused liver. Biochem. J. 102:943.

48) Sokal, J., 1966. Glucagon -- an essential hormone. Am. J. Med. 41:331.

49) Mallette, L.E., J.H. Exton, and C.R. Park, 1969. Control of gluconeogenesis from amino acids in the perfused rat liver. J. Biol. Chem. 244:5713.

50) Garcia, A., Williamson, J.R., and G.F. Cahill, 1966. Studies on the perfused rat liver. II. Effect of glucagon on gluconeogenesis. Diabetes 15:188.

51) Fiser, R.H., P.R. Williams, D.A. Fisher, P.V. DeLameter, et al, 1975. The effect of oral alanine on blood glucose and glucagon in the human newborn infant. Pediatrics 56:78.

52) Mallette, L.E., J.H. Exton, and C.R. Park, 1969. Effects of glucagon on amino acid transport and utilization in the perfused rat liver. J. Biol. Chem. 244:5724.

53) Landau, R.L., and K. Lugibihl, 1969. Effect of glucagon on concentration of several free amino acids in plasma. Metabolism 28:265.

54) Felig, P., W.V. Brown, R.A. Levine, and G. Klatskin, 1970. Glucose homeostasis in viral hepatitis. N. Engl. J. Med. 283:1436.

55) Muller, W.A., G. Faloona, and R.H. Unger, 1971. Effect of alanine on glucagon secretion. J. Clin. Invest. 50:2215.

56) Wise, J.K., R. Hendler, and P. Felig, 1972. The glycemic response to alanine: index of glucagon secretion in man. Clin. Res. 20:561.

57) Luck, J.M., G. Morrison, and L.F. Wilbur, 1928. The effect of insulin on the amino acid content of blood. J. Biol. Chem. 77:151.

58) Levine, R., and Fritz, I.B., 1956. The relation of insulin to liver metabolism. Diabetes 5:209.

59) Sinex, F.M., J. MacMullen, and A.B. Hasting, 1952. The effect of insulin on the incorporation of C^{14} into the protein of the rat diaphragm. J. Biol. Chem. 198:615.

60) Felig, P., and J. Wahren, 1971. Influence of endogenous insulin on splanchnic glucose and amino acid metabolism. J. Clin. Invest. 50:1702.

61) Sladek, C.D., and J.F. Snarr, 1974. Concentration dependent inhibition of hepatic gluconeogenesis by insulin. Proc. Soc. Exp. Biol. and Med. 146:194.

62) Buckman, M.T., M.J. Conway, J.A. Seibel, and R. Eaton, 1973. Effect of fasting on alanine-stimulated insulin and glucagon secretion. Metabolism 22:1253.

63) Hahn, P., 1975. Nurture of the newborn. New Eng. J. Med. 292:642.

64) Friedrichs, D., and W. Schoner, 1974. Regulation of gluconeogenesis by alanine. Bio. et Biophys. Acta 343:341.

65) Sladek, C.D., and J.F. Snarr, 1971. Effect of the exogenous amino acid concentration on the rate of gluconeogenesis in liver slices. Proc. Soc. Exp. Biol. Med. 138:181.

66) Rudorff, K.H., G. Albrecht, and W. Staib, 1970. Effect of insulin and proinsulin on the metabolism of alanine in the rat liver. Horm. Metabl. Res. 2:49.

67) Kirby, L., P. Hahn, 1974. Enzyme responses to prednisolone and dibutyryl adenosine 3', 5'-monophosphate in human fetal liver. Pediatr. Res. 8:37.

68) Cornblath, M., S.H. Wybregt, G.S. Baens, R.I. Klein, 1964. Symptomatic neonatal hypoglycemia: Studies of carbohydrate metabolism in the newborn infant VIII, Pediatrics 38:388.

69) Novak, M., and E. Monkus, 1973. The metabolism of subcutaneous adipose tissue in the immediate postnatal period of human neonates. III. Role of fetal glycogen in lipolysis and fatty acid esterification in the first hours of life. Pediat. Res. 9:769.

70) Shafir, E., and S. Kerpel, 1964. Fatty acid esterification and release as related to the carbohydrate metabolism of adipose tissue: Effect of epinephrine, cortisone and adrenalectomy. Arch. Biochem. Biophys., 105:237.

71) Novak, M., and E. Monkus, 1972. Metabolism of subcutaneous adipose tissue function in the immediate postnatal period of human newborns. I. Developmental changes in lipolysis and glycogen content. Pediat. Res. 6:73.

72) Pildes, R.S., R.S. Ramamurthy, and D.A. Patel, 1974. Diminished response to glucagon in the dextrose-primed neonate. Am. J. Dis. Child. 127:333.

73) Bloom, S.R., and D.I. Johnston, 1972. Failure of glucagon release in infants of diabetic mothers. Brit. Med. J. 4:453.

74) Rudolph, A.J., et al, 1959. Early versus late feeding of infants of diabetic mothers: a controlled study. J. Dis. Child. 98:496.

75) Santeusania, F., G.R. Faloona, and R.H. Unger, 1973. Inhibition of alanine-stimulated glucagon secretion by secretin in the dog. Horm. Metab. Res. 5:425.

76) Unger, R.H., 1971. Glucagon physiology and pathophysiology. N. Engl. J. Med. 285:443.

SOURCES CONSULTED BUT NOT CITED SPECIFICALLY

Desmond, M.M., 1953. Observations related to neonatal hypoglycemia. J. Pediat. 29:253.

Farquhar, J.W., 1954. Control of the blood sugar level in the neonatal period. Arch. Dis. Child. 519.

Felig, P., 1972. Interaction of insulin and amino acid metabolism in the regulation of gluconeogenesis. Israel J. Med. Sci. 8:263.

Felig, P., E. Marliss, 1972. The glycemic response to arginine in man. Diabetes 21:308.

Fernandes, J., and W. Blom, 1974. The intravenous l-alanine tolerance test as a means for investigating gluconeogenesis. Metabolism 23:1149.

Genuth, S.M., and J. Castro, 1974. Effect of oral alanine on blood betahydroxybuturate and plasma glucose, insulin, free fatty acids, and growth hormone in normal and diabetic subjects. Metabolism 23:375.

Haworth, J.C., F.J. Coodin, K.C. Finkel, M.L. Weidman, 1963. Hypoglycemia associated with symptoms in the newborn period. Canad. Med. Assoc. J. 88:23.

Isles, T.E., M. Dickson, and J.W. Farquhar, 1968. Glucose tolerance and plasma insulin in newborn infants of normal and diabetic mothers. Pediat. Res. 2:198.

Isles, T.E., and J.W. Farquhar, 1967. The effect of endogenous antibody on insulin-assay in the newborn infants of diabetic mothers. Pediat. Res. 1:110.

McKittrick, J.B., 1950. Serial blood sugar determinations in normal newborn infants. J. Pediat. 16:151.

Neligen, G.A., E. Robson, and J. Watson, 1963. Hypoglycemia in the newborn. Lannet 1:1282.

Oh, W., M.E. D'Amodio, L.L.Yap, and L. Hohenauer, 1970. Carbohydrate metabolism in experimental intrauterine growth retardation in rats. Am. J. Ob. Gyn. 108:416.

Ozand, P.T., J.T. Tildon, R.A. Wapnir, and M. Cornblath, 1973. Alanine formation by rat muscle homogenate, Biochem. Biophys. Res. Comm. 53:251.

Pederson, J., and M. Osler, 1961. Hyperglycemia as the cause of the characteristic features of the foetus and newborn of diabetic mothers. Dan. Med. Bull. 8:78.

Reisner, H., J.V. Aranda, E. Colle, D. Schiff, C. Scriver, and L. Stern, 1972. Effects of blood glucose, plasma insulin, growth hormone and alanine of different doses of glucagon on the first and third day of life. Israel J. Med. 6:791.

Steinke, J., S.G. Driscoll, 1965. The extractable insulin content of pancreas from fetuses and infants of diabetic and control mothers. Diabetes 14:573.

Wise, J.K., R. Hendler, and P. Felig, 1973. Evaluation of alpha-cell function by infusion of alanine in normal, diabetic and obese subjects. N. Engl. J. Med. 288:487.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE
Gary Gruppioni 370 Whitney Ave. N.H.	2/28/72